

1 Note

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3 **Black queen cell virus and drifting of honey bee workers**
 4 **(*Apis mellifera*)**

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7 **Gina Retschnig ^{a,b,*}, Liv A. Kellermann ^{a,b,c}, Marion M. Mehmman ^{a,b}, Orlando**
 8 **Yañez ^{a,b}, Pius Winiger ^{a,b}, Geoffrey R. Williams ^{a,b,d}, Peter Neumann ^{a,b}**

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11 ^a Institute of Bee Health, Vetsuisse Faculty, University of Bern,
 12 Schwarzenburgstrasse 161, 3003 Bern, Switzerland

13 ^b Agroscope, Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern,
 14 Switzerland

15 ^c Department of Agronomy, Bern University of Applied Sciences HAFL, Länggasse
 16 85, 3052 Zollikofen, Switzerland

17 ^d Department of Entomology & Plant Pathology, Auburn University, 301 Funchess
 18 Hall, Auburn, AL 36849, USA

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20

21 *Correspondence: E-mail: gina.retschnig@vetsuisse.unibe.ch,

22 Tel.: +41 (0)31 631 57 68

23 ORCID ID: 0000-0001-6249-9853

24 **Black queen cell virus (BQCV) / *Apis mellifera* / drifting / Virus**

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30 **Summary**

31 Social insects may accidentally drift into foreign nests due to orientation errors. Even
32 though pathogens have been reported to promote drifting, no data currently exist about
33 the potential impact of titers of the widespread black queen cell virus (BQCV) on the
34 orientation abilities of honey bee workers, *Apis mellifera*. Here, we investigated titers
35 of BQCV in naturally infected drifted and non-drifted workers. The data show
36 significantly higher virus titers in the drifted workers (Wilcoxon rank sum test, $P <$
37 0.01). Our results suggest that high BQCV loads may compromise honey bee
38 orientation, possibly by affecting learning performance similar to other viruses. If
39 future work demonstrates that the correlation found here represents a causal
40 relationship between higher viral titers and drifting, this will be the first identification
41 of clinical symptoms of BQCV in adult honey bee hosts.

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Honey bees may not return to their home colonies due to orientation errors, which is known as drifting (Rauschmayer 1928; Neumann et al. 2000). Infections with pathogens might enhance chances of orientation errors, thereby promoting both drifting and pathogen transmission between colonies (Fries and Camazine 2001). While honey bee viruses are common (Chen and Siede 2007), no significant association between the presence of ten common viruses and drifting of honey bee workers has been reported yet (Forfert et al. 2015). However, there may be an important difference with respect to phenotype between the sheer detection of a pathogen vs. the actual pathogen load, i.e. only higher virus loads may affect honey bee behavior as in case of learning and deformed wing virus (Iqbal and Mueller 2006). Here, we compared for the first time natural infection levels with the nearly ubiquitous black queen cell virus (BQCV) between drifted and non-drifted honey bee workers.

For this study, freshly emerged honey bee workers from four local colonies were individually marked and randomly introduced into three queenright 2-frame observation hives (N=200 each) that were installed in one row at intervals of ~1.5 m; each was equipped with an optically distinguishable hive entrance. All labelled workers that were present in the hives after 14 days were recaptured (N=178) and assigned to drifting status based on their markings (drifted N=13; non-drifted N=165). Then, BQCV levels were quantified using standard qPCR (Gauthier et al. 2007). Briefly, total RNA of individually homogenized workers was extracted using the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) and reverse transcription was conducted with the Thermoscript™ RT system (Invitrogen, Carlsbad, USA) according to manufacturer guidelines. qPCR was then performed using a KAPA SYBR FAST Universal Mastermix kit (KAPA Biosystems, Wilmington, USA) in an Eco™ Real

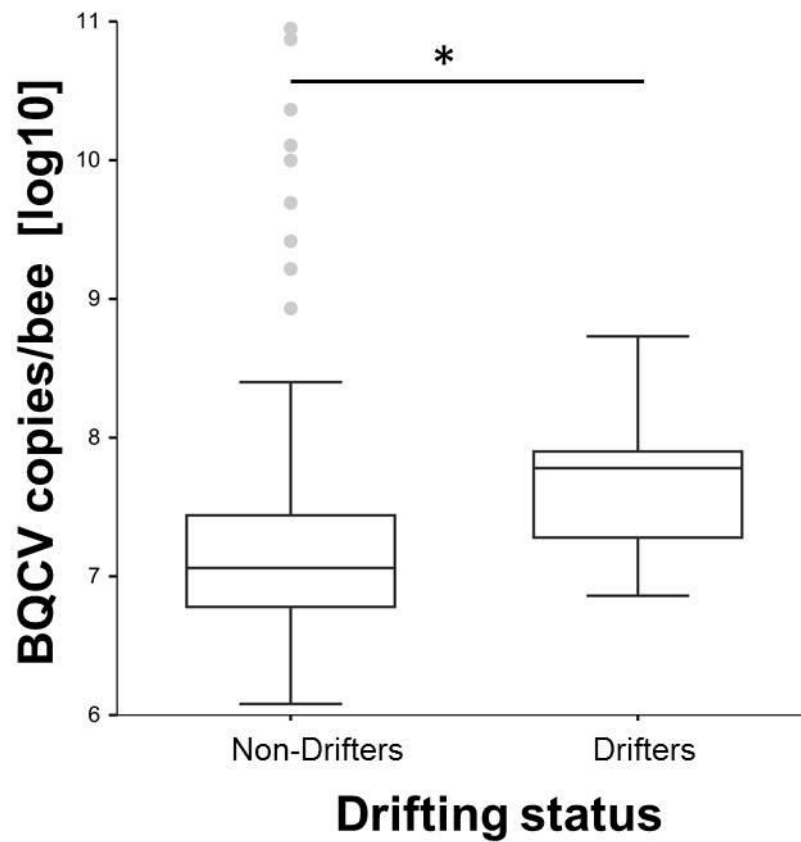
Time PCR System (Illumina, San Diego, USA) (de Miranda et al. 2013) with specific primers for BQCV published in Gauthier et al. 2007.

The analyses showed a significant effect of the observation hive on drifting ($\chi^2 = 10.52$, $P < 0.01$): 15.39 %, 4.62 % and 0 % of the bees drifted from hive 1, 2 and 3, respectively. Despite the limited sample size of drifting workers (N=13), they showed significantly higher BQCV titers compared to non-drifting ones (N = 165, Wilcoxon rank sum test, $P < 0.01$) (Figure 1).

In a previous study, no significant differences in the prevalence of BQCV and nine other common viruses were found between drifted and non-drifted honey bee workers (Forfert et al. 2015). However, the significantly higher viral titers of drifted workers in our study suggest that only high BQCV loads may compromise orientation abilities of their honey bee hosts, very similar to effects on learning performance by deformed wing virus (Iqbal and Mueller 2006). If that holds true, this compromised orientation ability would constitute the first reported clinical symptom of BQCV in adult honey bees (Chen and Siede 2007).

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90 **Figure I.** Drifting of adult worker honey bees and BQCV copies/bee. Drifted workers
 91 (N = 13) showed significantly higher BQCV titers compared to non-drifted ones
 92 (N = 165; Wilcoxon rank sum test, $P < 0.01=*$). Boxplots show the inter-quartile range
 93 (box), median (black line within box), data range (vertical lines) and outliers (grey
 94 dots). NCSS 10 was used for statistical analyses and the Figure.

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